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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

27 SEP 2004

Applicant's or agent's file reference PCB/SN/P89091WO	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB 03/01369	International filing date *(day/month/year)* 28.03.2003	Priority date *(day/month/year)* 28.03.2002
International Patent Classification (IPC) or both national classification and IPC A61K31/365		
Applicant UNIVERSITY OF LIVERPOOL et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, [illegible]gs which have been amended and are the basis for this report and/or sheets containing rect[illegible] this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under t[illegible]

   These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
12.09.2003	09.02.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Albayrak, T Telephone No. +49 89 2399-7549

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB 03/01369

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-58 as originally filed

Claims, Numbers

1-30 received on 21.01.2004 with letter of 19.01.2004

Drawings, Sheets

1/20-20/20 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

   see separate sheet

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	3-6, 10, 14-22, 27-30 (amended claims) 30-33 (original claims)
	No:	Claims	1, 2, 7-9, 11-13 (amended claims)
Inventive step (IS)	Yes:	Claims	3, 5, 6, 10, 16, 17, 19-22, 27-30 (amended claims)
	No:	Claims	4. 14, 15, 18 (amended claims) 30-33 (original claims)
Industrial applicability (IA)	Yes:	Claims	1-22, 27-30 (amended claims) 30-33 (original claims)
	No:	Claims	

2. Citations and explanations

   see separate sheet

Re Item I

The basis of this report is the following:

Description, pages:

1-58 as originally filed

Claims, No.:

1-30 as received on 21/01/2004 with letter of 19/01/2004

Drawings, sheets:

[illegible] as originally filed

Amended claim 23 appears not to be in line with Art. 34(2)b PCT.
This claim is directed to a method of screening compounds, to test whether or not said compounds have efficacy for use in chemotherapy, comprising 2 independent steps. First step is the exposition of the compounds to Topoisomerase II (Topo II), second step the exposition of the compounds to Heatshock Protein 90 (Hsp 90). Compounds are selected, which have binding capacity to both proteins.

The relevant passage in the application as originally filed is on page 39, first full paragraph. The described screening method comprises only one step which is the exposition of compounds to Topo II AND Hsp 90 to evaluate whether or not said compounds prevent interaction between Topo II and Hsp 90. It is not necessary for a target compound to be able to bind to both proteins independently to prevent interaction.

No further passage in the application as originally filed can be found which could serve as a basis for the subject-matter of amended claim 23.

Claims 24-26 are depended on claim 23 and therefore also seem to have no basis in the application as originally filed.

Therefore it appears, that claims 23-26 do not meet the criteria of Art. 34(2)b PCT.

The examination is carried out, as if the amendments for original claim 30 would not have been made.

Item V

1) Reference is made to the following documents; unless otherwise indicated, reference is made to the relevant passages emphasized in the Search Report.

D1: BARKER CR; RACKSTRAW S; HAMLETT J; PENNINGTON SR; WATSON AJM; JENKINS JR: 'Topoisomerase II associated proteins in colon cancer cells', GUT, 01.04.2003, page A56

D2: MÜNSTER PN; BASSO A; SOLIT D; NORTON L; ROSEN N: 'Modulation [illegible] by ansamycins sensitizes breast cancer cells to chemotherapy-induced apop[illegible] schedule-dependent manner', CLINICAL CANCER RESEARCH, 01.08.200[illegible]2228 to 2236

D3: BLAGOSKLONNY MV; FOJO T; BHALLA KN; KIM J-S; TREPEL JB; FIGG WD; RIVERA Y; NECKERS LM: 'The Hsp90 inhibitor geldanamycin selectively sensitizes Bcr-Abl-expressing leukemia cells to cytotoxic chemotherapy', LEUKEMIA, 2001, vol. 15, pages 1537 to 1543

D4: BLAGOSKLONNY MC: 'Hsp-90-associated oncoproteins: multiple targets of geldanamycin and its analogs', LEUKEMIA, April 2002, vol. 16, pages 455 to 462

D5: NECKERS L: 'HSP90 inhibitors as novel cancer chemotherapeutic agents', TRENDS IN MOLECULAR MEDICINE, 2002, vol. 8, no. 4, pages 55 to 61

The following document was cited by the Examiner:

D6: LEYCA A; PESSOA C; BOOGAERDT F; SOKAROSKI R; LEMONS TLG; WETMORE LA; HURUTA RR; MORAES MO: 'Oncocalyxones A and C, 1,4-Anthracenediones from Auxemma oncocalyx: Comparison with Anticancer 1,9-Anthracenediones', ANTICANCER RESEARCH, 2000, vol. 20, pages 1029-1032

D7: BINASCHI M; BIGIONI M; CIPOLLONE A; ROSSI C; GOSO C; MAGGI CA; CAPRANICO G; ANIMATI F: 'Anthracyclines: Selected New Developments', CURRENT MEDICAL CHEMISTRY, 2001, vol. 1, no. 2, pages 113-130.

2) **Novelty**

The subject-matter of amended claims 1, 2, 7-9, 11-13 is not novel.

D6 discloses 1,9-Anthracenediones and discusses the mechanisms of action in therapy. Among the disclosed compounds Doxorubicin is cited as being an 1,9-Anthracenedione which acts as an inducer of DNA strand breaks through interaction with topoisomerase II (see page 1029, right-hand column, 1st full paragraph and page 1031, left-hand column, lines 16-18).

D2 discloses the use of 17-allyl-aminogeldanamycin (17-AAG) which is an ansamycin in combination with doxorubicin, which is a dioxoanthracene, for the treatment of breast cancer cells.
D5 discloses the use of 17-allyl-aminogeldanamycin (17-AAG) in combination with doxorubicin.
The authors showed a significant synergistic [illegible] of the cytotoxicity of doxorubicin in the presence of 17-AAG. [illegible] known to inhibit Topo II. Therefore it appears, that the subject-matter of claims 1,2,7,9,11,12,13 does not meet the criteria of Art. 33 (2) PCT.

D3 discloses the use of Geldanamycin (GA) which is an ansamycin in combination with doxorubicin, which is a dioxoanthracene, for the treatment of cancer cells. The authors showed a significant increase of the cytotoxicity of doxorubicin in the presence of GA. Doxorubicin is known to inhibit Topo II. Therefore the subject-matter of claims 1,2,7,8,11,12,13 does not meet the criteria of Art. 33 (2) PCT.

3) **Inventive step**

The subject-matter of amended claims 4, 14, 15, 18 and original claims 30-33 is not inventive.

a) The subject-matter of claims 14-15 is the use of a first agent that attenuates Topoisomerase II (Topo II) activity and a second agent that inhibits Heat Shock Protein 90 (HSP 90) activity in the manufacture of a medicament for the treatment of paediatric tumours, neuroblastoma, leukaemias and lymphomas.
The treatment of cancer cells by the administration of a Topo II inhibitor and a

HSP90 inhibitor is already known from the prior art (see D2, D3 and D5). The specification of different tumors in claims 14-15 doesn't lead to any unexpected/surprising effect.

Claim 18 is directed to the use of a first agent that attenuates Topoisomerase II (Topo II) activity and a second agent that inhibits Heat Shock Protein 90 (HSP 90) activity in the manufacture of a medicament for prophylactic treatment.
The treatment of cancer cells by the administration of a Topo II inhibitor and a HSP90 inhibitor is already known from the prior art (see D2, D3 and D5). The feature "prophylactic" doesn't lead to any surprising/unexpected effect.

b) The underlying problem of original claims 30-33 is the provision of screening methods for the identification of inhibitors which inhibit the interaction of Hsp90 and Topo II. The solution, according to the applicant, lay in the provision of screening methods as claimed in original claims 30-33.
The screen is carried out by exposing compounds to Topo II and Hsp90 and to evaluate whether the compounds prevent interaction between Topo II and Hsp90 and thereby destabilize Topo II, since Hsp90 is known to be a chaperone.
Compounds which are Hsp90 inhibitors are already known from the prior art (see for example D2, page 2228, right-hand column, Introduction and page 2231, Figure 3B) and can be screened by methods which are known to those skilled in the art, for example by detecting the activity of a target protein of Hsp90. For this purpose Raf or HER2 could be used as target proteins (see: D2, page 2234, right-hand column, lines 38-42). Inhibitors of Hsp90 are known to decrease the intracellular concentration of targetproteins of Hsp90 which are involved in the development of cancer and that the antitumoral effect of Hsp90 inhibitors depends on the destabilization of these targetproteins. Therefore it appears, that the selection of Topo II as a target protein of Hsp90 is an arbitrary selection of possible target proteins and that the claimed screening method lacks an inventive step, since agents, which are known to inhibit Hsp90 are already known from the prior art and are used to decrease the activity of every protein which is known to be stabilized by Hsp90.
In the case, that the selection of Topo II as a target protein of Hsp90 would lead in the claimed screening method to specific inhibitors of Hsp90 which specifically inhibit the interaction of Topo II and Hsp90, it would be doubtful, whether the claimed compounds of claim 1 solve the problem (specific decrease of the activity

of Topo II via the inhibition of Hsp90) over the whole of the claimed scope. Especially, since 17-AAG was used to decrease targetproteins of Hsp 90 (see D2, Figure 3). In this case the application would not meet the requirements of Art. 6 PCT.

c) D2, D3 and D5 disclose the synergistic activity of Doxorubicin with 17-AAG or GA.
Claim 4 differs from D2, D3 and D5 in that Mitoxantrone was chosen as the compound which inhibits Topo II.

D7 discloses a study on the mechanism of action of different Anthracyclines. The authors state, that the antitumoral activity of anthracyclines lies in the stabilization of a Topo II - DNA complex which leads to DNA fragmentation (see page 113, right-hand column, 1st full and 2nd paragraphs).

the fact, that Mitoxantrone as well as Doxorubicin are both 1,9-anthracenediones, they're also regarded as being anthracyclines.
Since the mechanism of action between the two compounds are the same the skilled person would undoubtedly come to the subject-matter of claim 4 after combining D7 and D2 or D3 or D5.

CLAIMS

1. A use of a first agent that attenuates Topoisomerase II (Topo II) activity and a second agent that inhibits Heat Shock Protein 90 (HSP90) activity in the manufacture of a medicament for contemporaneous or sequential administration in chemotherapy wherein the first agent is selected from:

a Podophyllotoxin and derivatives and analogues thereof;
an Anthracenedione and derivatives and analogues thereof;
m-AMSA (amsacrine) and derivatives and analogues thereof;
a Bisdioxopiperazine and derivatives and analogues thereof.
a thiobarbiturate
Genistein and derivatives or analogues thereof; or
Pyrazoloacridine and derivatives or analogues thereof.

2. The use according to claim 1 wherein the first agent is a compound selected from:

(i) compounds that bind to Topo II and inhibit its activity (e.g. competitive inhibitors or allosteric inhibitors);

(ii) compounds which prevent the transcription, translation or expression of Topo II (e.g. ribozymes or antisense DNA molecules);

(iii) compounds which inhibit release of Topo II from intracellular stores; and

(iv) compounds which increase the rate of degradation of Topo II.

3. The use according to claim 1 or 2 wherein the first agent is a Podophyllotoxin and derivatives and analogues thereof and is selected from the group consisting of etoposide (VP16) or teniposide.

4. The use according to claim 1 or 2 wherein the first agent is the Anthracenedione Mitoxantrone.

5. The use according to claim 1 or 2 wherein the first agent a Bisdioxopiperazine and derivatives and analogues thereof and is selected from the group consisting of ICRF-154, 159, 187 or 193.

6. The use according to claim 1 or 2 wherein the first agent is the thiobarbiturate Merbarone or a derivative or analogue thereof.

7. The use according to any preceding claim wherein the second agent is a compound selected from:

(i) compounds that bind to Hsp90 and inhibit its activity (e.g. competitive inhibitors or allosteric inhibitors);

(ii) compounds which prevent the transcription, translation or expression of Hsp90 (e.g. ribozymes or antisense DNA molecules);

(iii) compounds which inhibit release of Hsp90 from intracellular stores; and

(iv) compounds which increase the rate of degr[illegible]sp90.

8. The use according to claim 7 wherein the s[illegible] is Geldanamycin or a derivative or analogue thereof.

9. The use according to claim 8 wherein the second agent is 17-Allylamino, 17-demethoxygeldanamycin (17AAG).

10. The use according to claim 7 wherein the second agent is Radicicol or a derivative or analogue thereof.

11. The use according to any preceding claim wherein the chemotherapy is for cancer treatment.

12. The use according to claim 11 for the treatment of solid tumours.

13. The use according to claim 12 for the treatment of bowel cancer, small cell and non-small cell lung cancer, head and neck cancer, breast cancer, bladder cancer or malignant melanoma.

14. The use according to claim 11 for the treatment of paediatric tumours.

15. The use according to claim 14 for the treatment of neuroblastoma, leukaemias and lymphomas.

16. The use according to claim 11 wherein the first agent is etoposide and it is used in the treatment of cancers selected from:

Adult Acute Myeloid Leukemia
Adult Hodgkin's Disease
Adult Non-Hodgkin's Lymphoma
AIDS-Related Lym[illegible]
Carcinoma of [illegible]
Childhood Acute [illegible]Leukemia
Childhood Brain Tumor
Childhood Cerebral Astrocytoma
Childhood Ependymoma
Childhood Hodgkin's Disease
Childhood Liver Cancer
Childhood Medulloblastoma
Childhood Non-Hodgkin's Lymphoma
Childhood Rhabdomyosarcoma
Childhood Supratentorial Primitive Neuroectodermal and Pineal Tumors
Childhood Visual Pathway and Hypothalamic Glioma
Endometrial Cancer
Ewing's Family of Tumors Including Primitive Neuroectodermal Tumor (PNET)
Extragonadal Germ Cell Tumors
Gastric Cancer
Gastrointestinal Carcinoid Tumor

Gestational Trophoblastic Tumor
Kaposi's Sarcoma
Malignant Thymoma
Neuroblastoma
Non-small Cell Lung Cancer
Osteosarcoma/Malignant Fibrous Histiocytoma of Bone
Ovarian Epithelial Cancer
Ovarian Germ Cell Tumor
Pediatric Extracranial Germ Cell Tumor
Prostate Cancer
Retinoblastoma
Small Cell Lung Cancer
Testicular Cancer
Unusual Cancers of Childhood
s' Tumor and Other Childhood Kidney Tumors

The use according to any one of claims 1 - 10 wherein the chemotherapy is for:

antibacterial treatments;
antifungal treatments;
the treatment of AIDS/HIV;
the treatment of multiple sclerosis; or
the killing and inhibition of proliferation of any organism.

18. The use according to any preceding claim wherein the chemotherapy is for prophylactic treatment.

19. A delivery system for use in a gene therapy technique, said delivery system comprising:

(i) a first DNA molecule encoding for a protein which directly or indirectly attenuates Topoisomerase II activity; and

(ii) a second DNA molecule encoding for a protein which directly or indirectly inhibits Heat Shock Protein 90 activity;

wherein said DNA molecules are capable of being transcribed to allow the expression of said proteins and thereby be effective for chemotherapy.

20. The use of a delivery system according to claim 19 for the manufacture of a medicament for use in chemotherapy.

21. The use according to claim 20 for the treatment of conditions defined by any one of claims 11 to 18.

22. A method of screening a first and a second compound, to test whether or not said compounds has efficacy for use in combination as a chemotherapy, comprising:

(a) exposing said compounds to Topoisomerase II and evaluating whether or not said compounds bind thereto;

(b) exposing said compounds to Heatshock Protein 90 and evaluating whether or not said compounds bind thereto; and

(c) selecting a first and second compound, wherein at least one compound binds to Topoisomerase II and at least one compound binds to Heatshock Protein 90 for use in combination as a chemotherapy.

23. A method of screening compounds, to test whether or not said compounds have efficacy for use in chemotherapy, comprising:

(d) exposing said compounds to Topoisomerase II and evaluating whether or not said compounds bind thereto;

(e) exposing said compounds to Heatshock Protein 90 and evaluating whether or not said compounds bind thereto; and

selecting compounds that bind to Topoisomerase II and to Heatshock Protein 90 for use in chemotherapy.

24. The method according to claim 22 or 23 wherein the compound is screened using Topoisomerase II and Heatshock Protein 90 as binding partners in an interaction trap and evaluating whether or not said compound modulates binding.

25. The method according to claim 24 wherein the interaction trap is a yeast two-hybrid interaction trap.

26. The method according to claim 25 wherein yeast used in the interact trap are permeable to the tested compounds.

27. A method of screening a compound, to test whether or not said compound is carcinogenic, comprising exposing said compound to Topoisomerase II and Heatshock Protein 90 to evaluate whether or not said compound promotes interaction between Topoisomerase II and Heatshock Protein 90.

28. An *in vitro* method for diagnosing whether [illegible] or is likely to develop cancer, comprising:

(i) detecting the level of activity or expression levels of HSP90 and Topoisomerase II from a sample of cells from said subject; and

(ii) comparing the level of activity or expression levels of HSP90 and Topoisomerase II in said sample relative to activity expression levels of HSP90 and Topoisomerase II from a non-cancerous sample.

29. An *in vitro* method for evaluating the suitability of chemotherapeutic treatment for administration to a subject, comprising:

(i) detecting the level of activity or expression levels of HSP90 and Topoisomerase II from a sample of cells from said subject; and

(ii) comparing the level of activity or expression levels of HSP90 and Topoisomerase II in said sample relative to activity expression levels of HSP90 and Topoisomerase II from a non-cancerous sample.

30. An *in vitro* method for monitoring the effectiveness of a chemotherapy for treating a subject, comprising:

(i) detecting the level of activity or expression levels of HSP90 and Topoisomerase II from a sample of cells from said subject; and

(ii) comparing the level of activity or expression levels of HSP90 and Topoisomerase II in said sample relative to activity expression levels of HSP90 and Topoisomerase II from a non-cancerous sample.